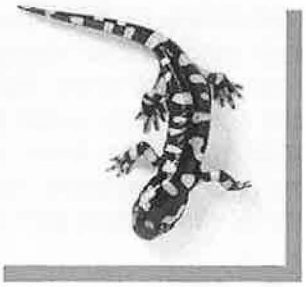
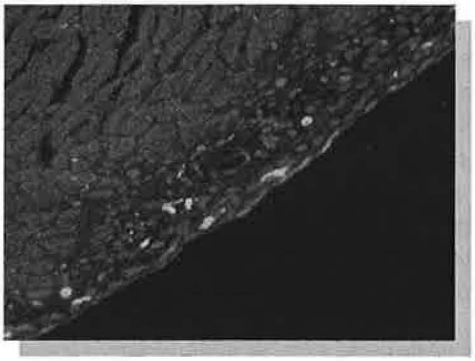
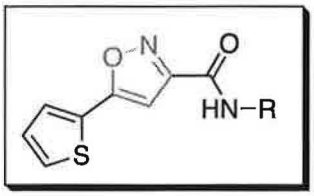
INTERNAL MEDICINE GRAND ROUNDS

October 24, 2008

Newts, niches, and new drugs: the next frontier for regenerative medicine







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Dr. Schneider has no financial interests or other relationships with commercial concerns related directly or indirectly to this program, and he will not be discussing off-label drug uses in this presentation.

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Stem/progenitor cell biology
Chemical biology
Regenerative Medicine
Cardiomyocyte biology

Introduction

Having successfully doubled his/her longevity over the last century, mankind has created the new problem of aging, extending the duration of life well beyond the teleological design limit. The trade-off for defeating our natural microbial adversaries through improved public health and hygiene, antibiotics, and vaccines, is an aging human population whose organ systems and tissues are burdened by degenerative changes from excessive wear-and-tear, fed by a dwindling atherosclerotic vascular supply.

Resident pools of somatic stem/progenitor cells, located in specialized areas or microenvironments called niches, are responsible for adult tissue maintenance and minor repairs. Stem/progenitor cell niches provide an extended lifetime warranty for aging tissues, but coverage is limited. Most notably, adult human stem/progenitor cells in the heart and brain are incapable of repairing tissue destruction from myocardial infarction or stroke; after catastrophic ischemic tissue injury, the function of these injured organs seldom if ever returns to normal levels. ***Enhancing the reparative function of stem/progenitor cell niches with drugs that target key constituents of these new cell-generating centers in vivo is the next frontier of regenerative medicine.***

This grand rounds presentation will first describe the scope of the problem, focusing on cardiovascular disease, the #1 cause of death and disability, worldwide. It will provide a teleological perspective for why mankind, unlike more primitive newts and zebrafish, finds his/herself in the predicament of an irreparable heart, incapable of rebuilding damaged muscle. This presentation will contrast the *promise* of using stem/progenitor cells for repair with the *problems*, highlighting the roadblocks that must be overcome before greater clinical success can be achieved. It will provide an update and status report on current cardiac stem/progenitor cell therapy clinical trials for myocardial infarction and heart failure, whose success has been disappointingly modest. To end, this presentation will briefly describe our laboratory's efforts to define new synthetic organic small molecules – attractive future drugs – selected and designed to recruit stem/progenitor cells into heart muscle rebuilding/repair pathways by educating fate decisions ex vivo or in vivo in the epicardial niche.

Scope of the problem: Stem cells needed to regenerate heart muscle

The cost of survival: from myocardial infarction to heart failure

The past thirty years have seen a decline in cardiovascular mortality rates due to thrombolytic therapy and preventive therapies like statins, and advances in emergency and acute coronary care (3, 4). Paradoxically, our success in treating myocardial infarction has created a new, escalating, and expensive epidemic of heart failure (5). The fundamental problem is the human myocardium's inability to repair damage by rebuilding muscle. The irreversible loss of cardiomyocytes results in severely diminished cardiac pump function. However, there are biologic models of more primitive organisms like the newt or zebrafish, which can repair/regenerate catastrophic traumatic experimental heart muscle injury (6).

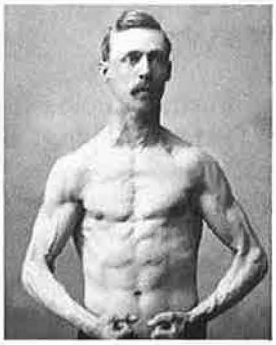
In discussing the mammalian heart, the word "*regeneration*" is a flashpoint for igniting heated dispute. On one side of the argument are scientists who challenge the age-old dogma that the mammalian heart is terminally differentiated. Driven by statistical analyses of cell counts from state-of-the-art immunohistochemical photomicrographs, these scientists claim the myocardium is perpetually renewing itself, constantly regenerating cardiomyocytes and other cells, generating a completely new heart as many as twenty times over a lifetime (7-9). If it didn't turnover, they argue, we'd be dead, because cardiomyocytes are constantly lost through apoptosis. The other extreme position is that the heart is a post-mitotic organ; you die with the same (or fewer) cardiomyocytes that you were born with, even if these muscle cells are lucky enough to survive 100 or more years of hard contractile labor.

The truth may lie somewhere in between these extremes (10). Recent lineage tracing studies (using ultra-modern and definitive genetic fate mapping tools) in mice confirmed that the injured adult mammalian heart can produce a limited number of new cardiomyocytes, but too few to restore normal contractile activity, and too slowly to counteract tissue replacement by heart-stiffening fibrosis (11). Importantly, the uninjured heart shows no detectable cardiomyocyte turnover (11).

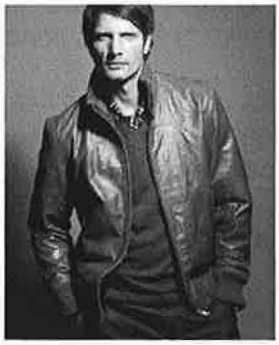
At the root of the human dilemma is our lack of knowledge regarding how to make new cardiomyocytes, one of the most structurally elegant and functionally complex (and hardest working) cells in nature, from undifferentiated progenitors. Despite a growing understanding of the core regulatory networks of embryonic growth factors, interacting transcription factors, and microRNAs that drive cardiac differentiation, mechanistic insight into the spark that triggers cardiac cell fate remains elusive (12, 13). More than twenty years after the discovery of the skeletal muscle master regulator MyoD by Weintraub and Lassar (14), we still lack a master regulator of cardiac muscle cell fate. New perspectives, experimental approaches, and scientific strategies are critically needed.

Cardiovascular disease, a man-made modern problem

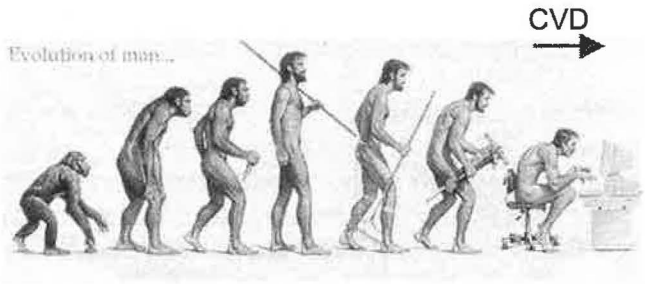
Why does modern man have the predicament of an irreparable heart? The answer begins with an epidemiologic review of health and disease during the last century[1]. In 1900, the average U.S. life expectancy at birth was 49.2 years; in 1970, it was 70.8 years. Among the key factors for enhanced life expectancy were the chlorination of municipal water supplies, penicillin, vaccination programs, and other public health measures, defeating microbes as mankind's greatest enemy. Yet, by the middle of the 20th century, the United States was a predominantly urban, industrial economy, with the majority of citizens living in burgeoning and polluted cities. With development of transportation systems, exercise for mobility became unnecessary, factory-rolled cigarettes were cheap and plentiful, and high fat, preservative-laden convenience food became commonplace and malnutrition rare. The middle of the 20th century has been called the age of degenerative and man-made diseases. Mankind had learned to live long and prosper. However, longer life takes a toll on hard-working tissues like the heart, which beats 35 million times/year – that's 20 extra years or uninterrupted beating. This puts a major burden on tissue repair mechanisms designed to maintain functional homeostasis. Extended human life expectancy in the context of an atherogenic environment and lifestyle places GQ man at risk for atherosclerotic cardiovascular disease, which is the greatest non-infectious health care problem ever to afflict mankind. Although some have argued, based on sophisticated imaging techniques, that frozen ice-age men and Egyptian mummies from thousands of years ago have evidence of atherosclerotic vascular disease, clearly the magnitude of problem would not compare to today's society.



Early 20th century man: life expectancy at birth ~49.2 years



Modern "GQ" man: life expectancy at birth ~70.8 years



Evolution of man: live long and prosper with enhanced risk of cancer, degenerative diseases, atherosclerosis, and tissue infarction. CVD= cardiovascular disease.

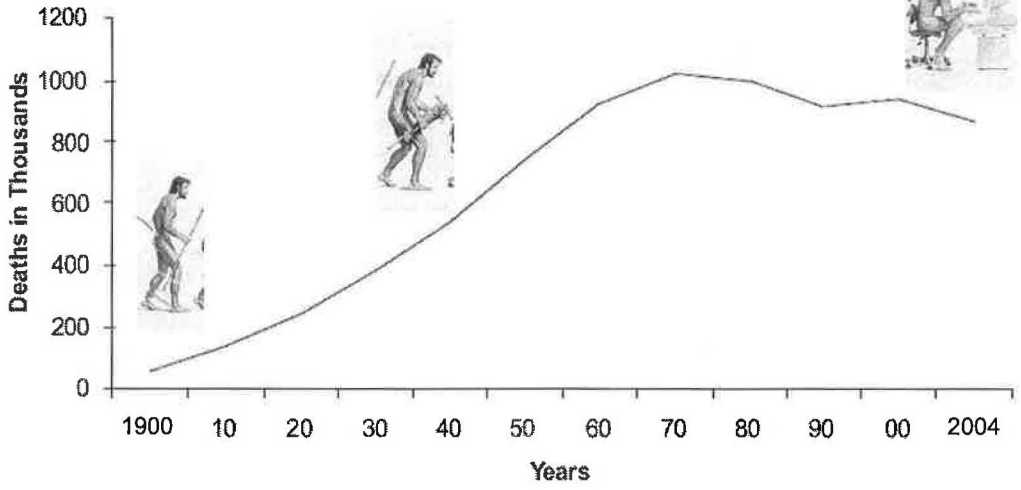


Chart 2-4. Deaths due to CVD (United States: 1900–2004). CVD does not include congenital HD.

[1] Global Burden of Cardiovascular Disease. J. Michael Gaziano, Chapter 1, pgs. 1-22, Braunwald's Heart Disease, 8th Edition, (P. Libby, R. Bonow, D. Mann, D. Zipes, eds.) 2008.

There is no natural mechanism for repairing injured myocardium to combat the wholesale loss of tissue that results from myocardial infarction. On the other hand, bacterial infections leading to endocarditis and valve dysfunction have existed for eons, in every species with a circulatory system, and the human heart has accordingly evolved sophisticated mechanisms of hypertrophy to deal with the volume or pressure overload that results from valve incompetence or stenosis. Faced with infarction, the human heart mounts a response characterized by a damage phase with persistent ischemia, clot formation, macrophage inflammatory reaction, cellular infiltrate for clearing debris, followed by a repair phase with cytokine-driven fibrosis. A rapid fibrotic patch is required to prevent rupture of the ventricle operating under high filling pressures. The heart's stem/progenitor cell niche plays an important role in this process but not for making new cardiomyocytes, which might restore function to normal by rebuilding muscle. The niche has insufficient resources and too little time to accomplish this, on it's own.

Lessons from the newt

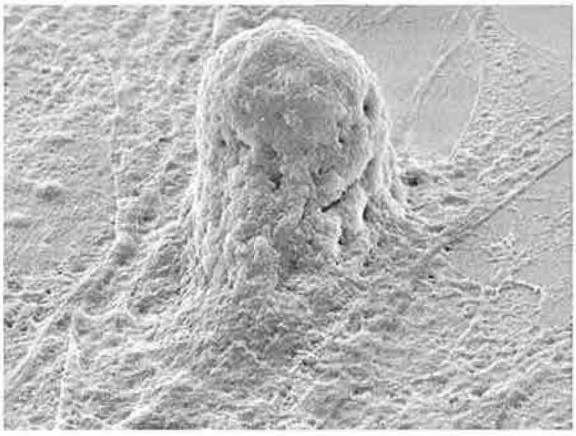
Myocardial infarction is event that only occurs in humans. The newt, on the other hand, has escaped the scourge of manmade atherosclerosis and organ infarction but lives in a competitive pond environment that shrinks in spring and summer, bringing these highly territorial animals closer and closer to neighbors, causing loss of limbs through vicious fighting. There is an evolutionary advantage to being able to rapidly re-grow the limb and re-establish dominance over the newt's territory. In experimental systems (when faced with the scientist's scissors), the newt's heart is the beneficiary of this conserved regenerative/repair mechanism which involves both dedifferentiation of mature cells to generate new precursor cells as well as pools of adult tissue stem/progenitor cells in the epicardium of the heart (6).

Increasing evidence indicates that the human adult epicardium, the highly specialized outermost layer of the heart abutting the myocardium and exposed to pericardial fluid, is the source of progenitors giving birth to new cardiomyocytes following injury (15, 16). The epicardial microenvironment is the heart's stem/progenitor cell niche. A better understanding of this niche, its intrinsic control mechanisms and how it responds to extrinsic cues, is essential. Even though the human cardiac stem/progenitor cell niche is incapable on its own of repairing an MI though muscle tissue regeneration, the basic mechanisms exist, providing the possibility that we might learn to therapeutically enhance its activity.

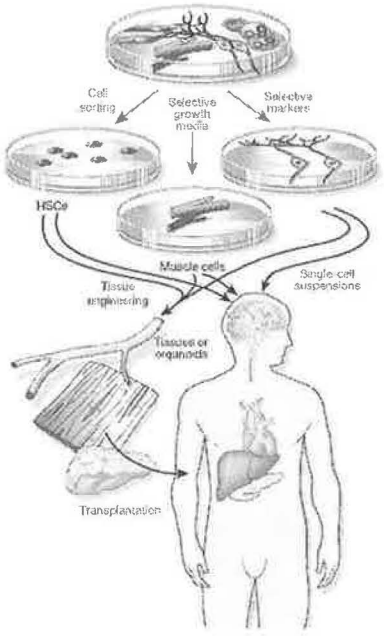
The Promise of Stem Cells

This year (2008) is the 10th year anniversary of the most famous statement regarding stem cells made by Dr. Harold Varmus, the Director of NIH, on the eve of Dr. James Thomson's 1998 report in *Science* of the first continuous lines of human pluripotent stem cells derived from IVF embryos (17). In his testimony to Congress regarding the importance and potential impact of this discovery, Dr. Varmus stated:

"***The development of cell lines that may produce almost every tissue of the human body is an unprecedented scientific breakthrough. It is not too unrealistic to say that this research has the potential to revolutionize the practice of medicine and improve the quality and length of life.***"[2]



Scanning electronmicroscopy photograph of a human embryonic stem cell attached to fibroblast feeder layer by Annie Cavanagh and Dave McCarthy, 2006 Wellcome Trust Biomedical Image Awards.



[2] http://www.hhs.gov/asl/testify/t981202a.html.

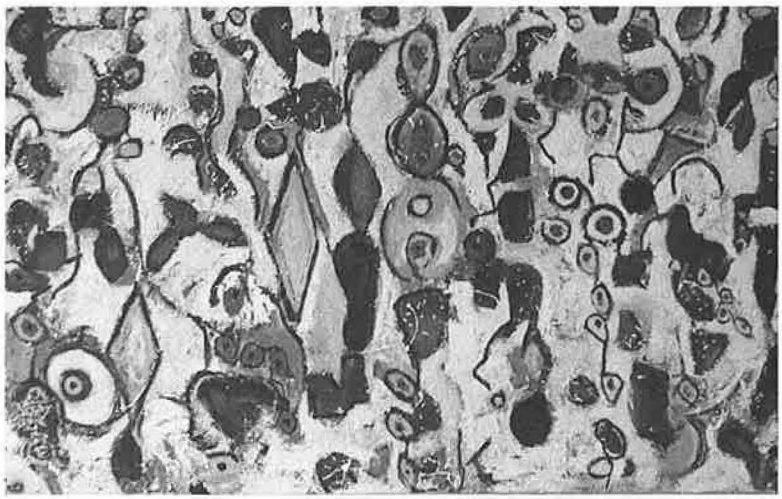
The problem with stem cells: too much potential, too little mechanistic insight

Stem cell snake oil

Beware of the stem cell snake oil salesman promising a cheap, cure-all elixir of everlasting life, warned an editorial entitled, "***The Stem-cell Sell,***" equating scientists clamoring for federal funds to the quintessential American huckster, the snake oil salesman[3].

To make this point, it is instructive to emphasize perhaps the greatest stem cell research success of the past ten years, a discovery that has eclipsed the importance of Thompson's human embryo-derived embryonic stem cells. The breakthrough came when Yamanaka and colleagues showed that adult mouse cells could be reprogrammed towards embryonic stem cells, generating "induced pluripotent state" stem cells (iPS cells), by introducing just four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc (18). The iPS technology is rapidly advancing with new strategies to omit oncogenes like c-Myc and integrated viral sequences from pluripotency cocktails, making these cells safer for future clinical applications (19, 20). Gene transfer strategies have also been coupled to small molecule chemical treatments that enhance the efficiency of the pluripotency conversion process (21, 22).

The future prospects for generating bankable individualized (patient-specific) iPS cells, unencumbered by most ethical issues, have taken a giant leap forward, yet what exactly we can accomplish with these cells clinically remains theoretical; they are dogged by the same scientific problems as human embryonic stem cells. ***Indeed, while iPS cells have changed everything, they've changed nothing, because the fundamental and monumental hurdle of controlling cell fate decisions remains a black box in iPS as in all other stem/progenitor cells.***



"Control of stem cell fate."

The apparent simplicity of tiny stem/progenitor cells with a large nucleus and a thin rim of pale undifferentiated cytoplasm is deceptive; these are extraordinarily complex and sophisticated cells. Stem/progenitor cells encompass a microcosm of all of molecular, cellular, and developmental biology. The high nuclear/cytoplasmic ratio of these cells underscores a fundamental biological principle: stem/progenitor cells do little else other than protect and manage their genome through complex and poorly understood epigenetic regulatory mechanisms.

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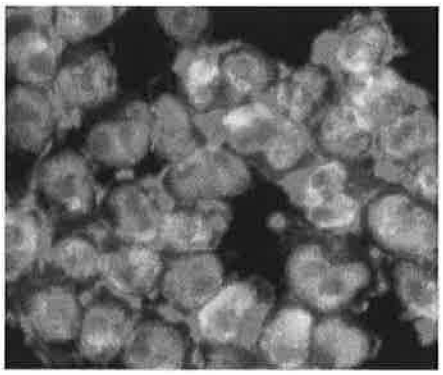
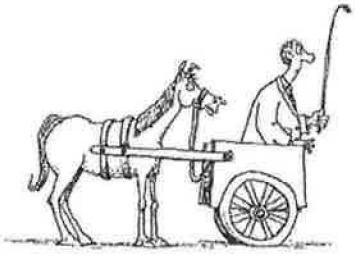
[3] The Stem-cell Sell. Editorial. Commonweal Magazine. August 17th, 2001, pg. 5; http://findarticles.com/p/articles/mi_m1252/is_/ai_78804059.



Photomicrograph of mouse P19 embryonal carcinoma stem/progenitor cells stained with an Oct3/4 antibody (green) and actin-binding phalloidin (orange) from our lab; note the high nuclear/cytoplasmic ratio.

Among the other issues that make stem/progenitor cells challenging targets for therapeutic exploitation is the fact that few drugs work in these cells, because they express elevated levels of drug efflux pumps. Moreover, traditional signal transduction cascades characteristic of their differentiated counterparts either are not expressed or are functionally uncoupled in these primitive cells.

Cardiac stem cell clinical trials: how did we start and where are we now?



There are two fundamentally opposing, yet equally defensible, viewpoints regarding cardiac stem cell clinical trials. The first one is that these trials are over-zealous and premature, placing the clinical cart before the scientific horse, and there are many serious potential dangers that we can't even anticipate yet. The second viewpoint is that, given the magnitude of the clinical problem, it would be unethical to delay clinical trials until we know the mechanism of action. Why should cardiac cell therapy be held to higher bench-to-bedside standard than small molecule pharmacologic therapeutic agents commonly used in clinical practice, where mechanisms are understood only partially, if at all?

From pioneer to plaintiff

U.S. teen 1st in world to get experimental stem cell heart treatment

2003. Doctors at Beaumont Hospital in Royal Oak, Michigan, are first in the world to use stem cells from a patient's own blood to try to repair damage caused by a heart attack. The patient, 16-year-old Dimitri Bonnville, had a massive heart attack in mid-February after being accidentally shot in the heart with a nail gun. The Almont, Michigan, native is recuperating at home. Doctors hope these stem cells will regenerate damaged heart tissue and stimulate the growth of new blood vessels.

The first clinical experience of cardiac stem cell transplant in the United States went from bedside-to-courtroom, bypassing the laboratory bench. In February 2003, prompted by stunningly successful pre-clinical studies in mice (1, 23), cardiologists at William Beaumont Hospital in Michigan made a daring clinical decision. A 16-year-old roughhousing teenager had been accidentally struck in the heart by a nail from a nail gun, the nail penetrated through the right ventricle into the left ventricle adjacent to the left anterior descending coronary artery. After trauma surgeons removed the nail, there was a massive myocardial infarction, causing severe systolic contractile dysfunction. This was deemed a mortal injury, yet the youth survived. The first choice of treatment was a heart transplant, but no donor heart was available. Instead, the family was offered a risky last-ditch experimental procedure. They would mobilize bone marrow stem/progenitor cells with hematopoietic growth factors, harvest bone marrow from the teenager's hip, wash the cells, and then re-inject them down the coronary arteries into the area of injury. At this time clinical trials with promising clinical results were underway abroad, in England, Germany, and Brazil, but not in the United States. The family accepted the risk and the procedure was done with fanfare (on TV) but without complication. Left ventricular performance improved almost immediately.

Despite the apparent success, the hospital and physicians were severely reprimanded by the FDA for undertaking a reckless human experiment without an approved protocol or legal informed consent. Even worse, lawyers convinced the family to have a "change of heart," converting the initial accolades for the daring physicians who "saved their son's life" into a multi-million dollar negligence lawsuit.

NHLBI directive

Now, five years later, the National Institutes of Health is providing unprecedented support for cardiac stem cell trials, believing this is an opportunity to dramatically alter the treatment of cardiovascular disease (24). In a recent NHLBI working group meeting, experts agreed to accelerate the process to clinical application, stating *three primary motivating forces*:

1. There is a compelling clinical need.

The clinical imperative is undeniable. Here are some of the most recent estimates (4):
For coronary disease:

- In 2008, ~770,000 Americans will suffer a new myocardial infarction
- In 2008, ~430,000 Americans will suffer a recurrent myocardial infarction
- In 2008, ~175,000 Americans will suffer a silent first myocardial infarction
- In 2008, there will be one myocardial infarction every 26 seconds, and one cardiac death every 60 seconds

For heart failure:

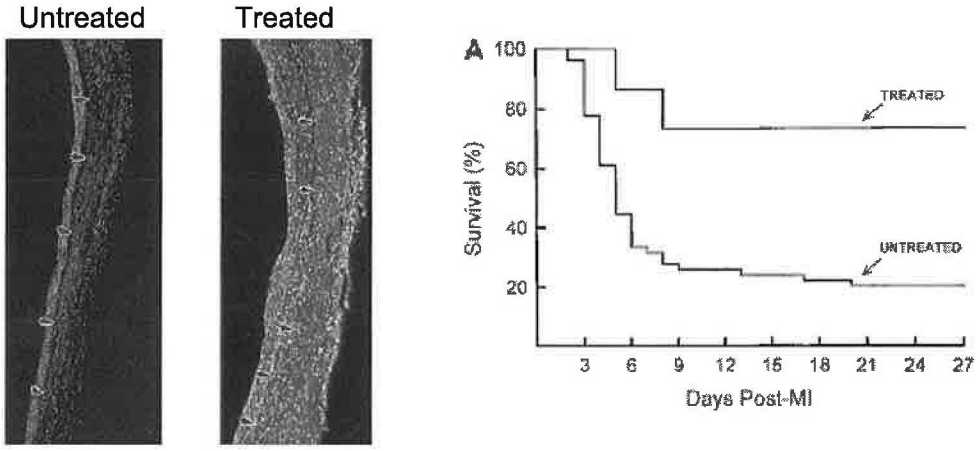
- The heart failure incidence is 1/100 population after age 65
- At age 40, lifetime risk for developing heart failure is 1/5.
- At diagnosis, the one-year heart failure mortality is 1/5.
- The estimated cost of heart failure in 2008 will be $34.8 billion

Thus, cardiovascular disease leading to heart failure is a devastating and expensive problem.

2. There is supportive preclinical data.

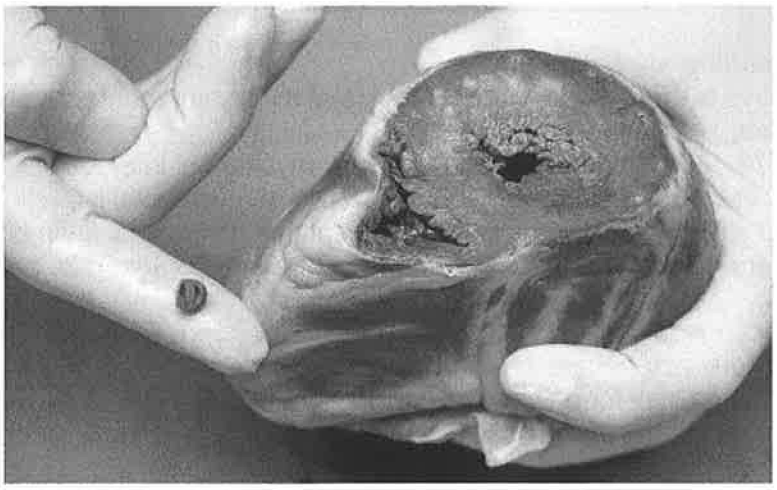
There have been a large number of preclinical studies, from around the world, addressing the effectiveness of cell transplantation or growth factor-mediated hematopoietic stem/progenitor cell mobilization in promoting heart repair/regeneration in animal models, mostly rodents. A large variety of stem/progenitor cell types have been explored, including embryonic stem cells, skeletal myoblasts,

mesenchymal stem cells, hematopoietic stem/progenitor cells, endothelial progenitor cells, epicardial-derived progenitor cells, and endocardial-derived progenitor cells, among others. Few studies report no improvement in ventricular function or survival, and there are numerous excellent very recent reviews (25, 26). To highlight just one study, one of the initial breakthroughs, Anversa and colleagues reported that pre-treatment of splenectomized mice G-CSF mobilized hematopoietic stem/progenitor cells that could repair an experimental myocardial infarction induced by ligation of the left anterior descending coronary artery and dramatically improve post-infarction survival (1). This study "rocked" the cardiology world, but could it really be that straightforward?



(Left hand panels) Photomicrographs of "regenerated" left ventricular myocardium after experimental infarction in control (untreated) mice or mice pre-treated with G-CSF (treated); blue staining shows fibrosis. (Right hand panels) Enhanced post-infarction survival in G-CSF pre-treated mice (1).

The mouse/human size differential:



3. Promising early clinical experience (27).

Early clinical trials have demonstrated safety but limited efficacy. However, even a few percentage points improvement in LVEF might be physiologically important and have long-term consequences for disease progression and survival.

Table 1. Randomized, Controlled Trials of BMC for Cardiac Disease.*

Trial or Investigator Group	Setting	Design	No. of Cells Administered in Treatment Group	Results
BOOST[4,9]	PCI after acute myocardial infarction	Randomized trial 30 patients received BMC; 30 received no infusion LVEF assessed by MRI	Approximately 2.5×10^9 unfractionated BMC	At 6 mo: LVEF 6% greater in BMC group than in control group At 18 mo: no significant difference in LVEF between the 2 groups
Janssens et al.[5]	PCI after acute myocardial infarction	Randomized, double-blind trial 33 patients received BMC; 34 received placebo infusion LVEF was assessed by MRI	Approximately 3×10^8 Ficoll-separated BMC	At 4 mo: no significant difference in overall LVEF; decreased infarct size and better regional function in BMC group
TOPCARE-CHD[6]	Chronic left ventricular dysfunction	Randomized, crossover trial In the second phase, 24 patients received CPC, 28 received BMC, 23 received no infusion LVEF assessed by left ventricular angiography	Approximately 2×10^8 Ficoll-separated BMC or approximately 2×10^7 Ficoll-separated, cultured CPC	At 3 mo: greater increase in LVEF (2.9 percentage points) in BMC group than in CPC group or control group
ASTAMI[7]	PCI after acute myocardial infarction	Randomized trial 47 patients received BMC; 50 received no infusion LVEF assessed by SPECT, echocardiography, and MRI	Approximately 7×10^7 Ficoll-separated BMC	At 6 mo: no significant difference in LVEF between the 2 groups
REPAIR-AMI[8]	PCI after acute myocardial infarction	Randomized, double-blind trial 101 patients received BMC; 98 received placebo infusion LVEF assessed by left ventricular angiography	Approximately 2.4×10^8 Ficoll-separated BMC	At 4 mo: greater absolute increase in LVEF in BMC group than in placebo group (5.5% vs. 3.0%) At 1 yr: reduction in combined adverse clinical events in BMC group as compared with placebo group

* BOOST denotes Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration, PCI percutaneous coronary intervention, MRI magnetic resonance imaging, TOPCARE-CHD Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease, CPC progenitor cells derived from circulating blood, ASTAMI Autologous Stem-Cell Transplantation in Acute Myocardial Infarction, SPECT single-photon-emission computed tomography, and REPAIR-AMI Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction.

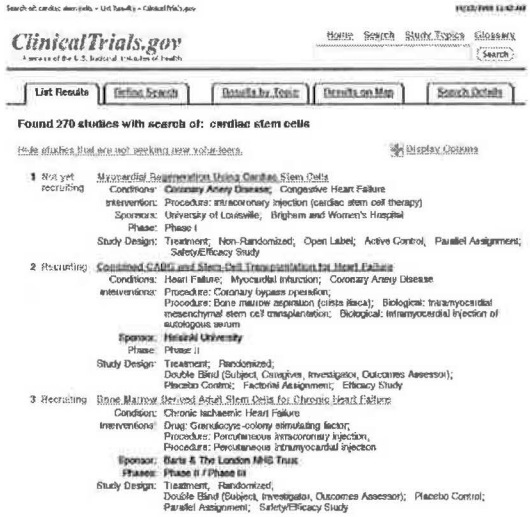
Controversy: why did REPAIR-AMI cells work but ASTAMI cells didn't?

Importantly, these trials have yet to definitively demonstrate the development of new cardiomyocytes or meaningful results. Very likely, despite injecting billions of stem/progenitor cells into these patient's hearts, not a single new cardiomyocyte has been produced. There are many technical issues regarding the follow-up of injected cells.

4. Additional reasons to proceed with US trials.

Dogged by ethical considerations, governmental regulations, and threat of malpractice lawsuits, we are trailing behind Europe and Asia in the race to clinic, even though we may lead the science.

Global explosion of new trials


ClinicalTrials.gov

Home Search Study Topics Glossary

List Results | Refine Search | Details by Topic | Results on Map | Search Details

Found 270 studies with search of: cardiac stem cells

Hide studies that are not seeking new volunteers. Display Options

1 Not yet recruiting — Myocardial Regeneration Using Cardiac Stem Cells
Conditions: Coronary Artery Disease; Congestive Heart Failure
Intervention: Procedure: intracoronary injection (cardiac stem cell therapy)
Sponsors: University of Louisville; Brigham and Women's Hospital
Phase: Phase I
Study Design: Treatment; Non-Randomized; Open Label; Active Control; Parallel Assignment; Safety/Efficacy Study

2 Recruiting — Combined CABG and Stem-Cell Transplantation for Heart Failure
Conditions: Heart Failure; Myocardial Infarction; Coronary Artery Disease
Interventions: Procedure: Coronary bypass operation; Procedure: Bone marrow aspiration (crista iliaca); Biological: Intramyocardial mesenchymal stem cell transplantation; Biological: Intramyocardial injection of autologous serum
Sponsor: Helsinki University
Phase: Phase II
Study Design: Treatment; Randomized; Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor); Placebo Control; Factorial Assignment; Efficacy Study

3 Recruiting — Bone Marrow Derived Adult Stem Cells for Chronic Heart Failure
Condition: Chronic Ischaemic Heart Failure
Interventions: Drug: Granulocyte-colony stimulating factor; Procedure: Percutaneous intracoronary injection; Procedure: Percutaneous intramyocardial injection
Sponsor: Barts & The London NHS Trust
Phases: Phase II / Phase III
Study Design: Treatment; Randomized; Double Blind (Subject, Investigator, Outcomes Assessor); Placebo Control; Parallel Assignment; Safety/Efficacy Study

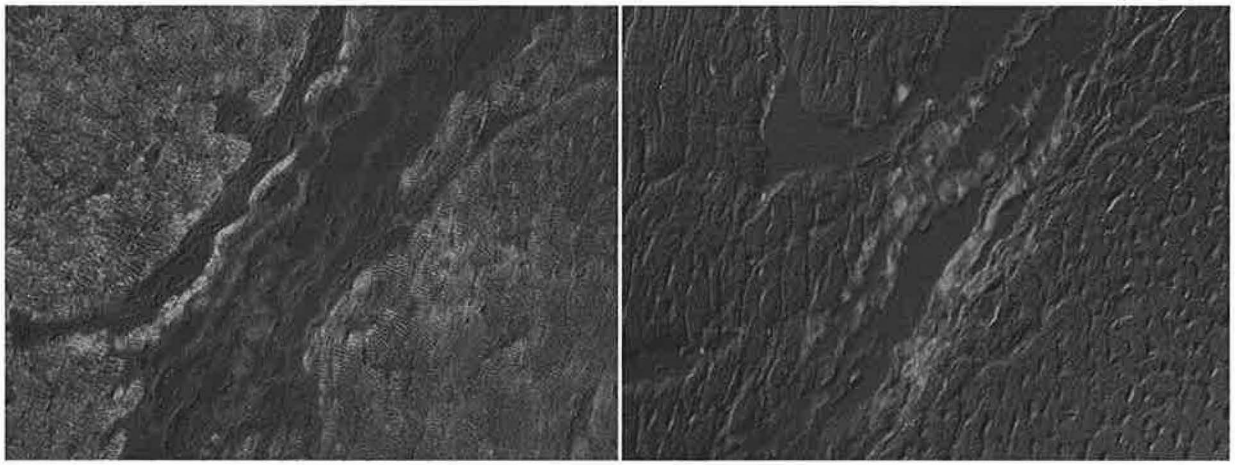
There has been an explosion of new studies, worldwide. Using "cardiac stem cells" as a keyword to search the NIH ClinicalTrials.gov webpage identifies 270 studies. The first ten are:

1. Myocardial regeneration using cardiac stem cells (phase I)
2. Combined CABG and stem cell transplantation for heart failure (phase II)
3. Bone marrow derived adult stem cells for chronic heart failure (phase II/phase III)
4. Prospective randomized study of mesenchymal stem cell therapy in patients undergoing cardiac surgery (PROMETHEUS) (phase I/phase II)

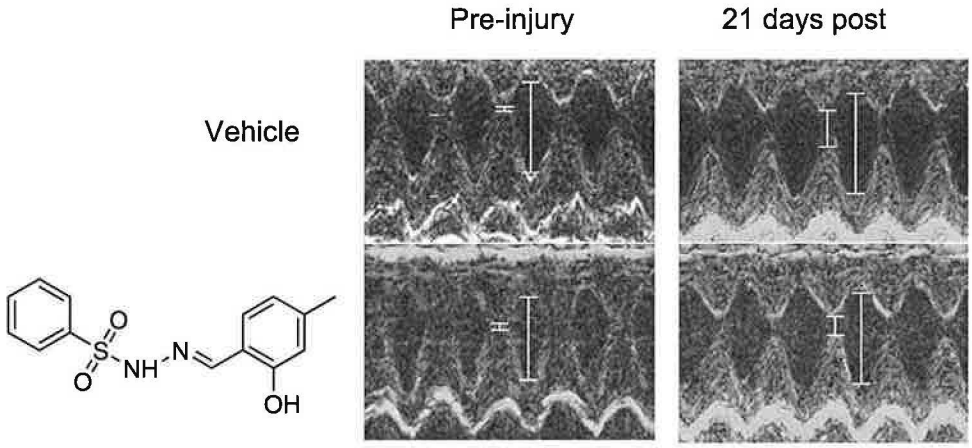
5. Stem cell mobilization to treat chest pain and shortness of breath in patients with coronary artery disease (phase II)
6. Study on the efficacy and mechanism of cardiac rehabilitation for stem cell mobilization and heart failure improvement
7. Intramyocardial injection of autologous aldehyde dehydrogenase-bright stem cells for therapeutic angiogenesis (phase I)
8. Safety and efficacy of autologous, intracoronary stem cell injections in total coronary artery occlusions (phase I)
9. Bone marrow derived adult stem cells for acute anterior myocardial infarction (phase II/phase III)
10. By pass surgery with stem cell therapy in chronic ischemic cardiomyopathy (phase II)

Our approaches

On their own, there is little hope that uneducated hematopoietic or mesenchymal stem/progenitor cells injected into the human heart can become functionally integrated cardiomyocytes. We have taken a chemical biology approach, designing pharmacotherapeutic strategies to improve stem/progenitor cell function in heart repair (28-30). In collaboration with Hesham Sadek (new cardiology faculty at UT Southwestern), we pre-treat hematopoietic stem/progenitor cells ex vivo with cardiogenic synthetic small molecules, chemically educating these cells in fate decisions. We identified our cardiogenic small molecules from a high throughput screen of the UT Southwestern organic chemical compound library (29).

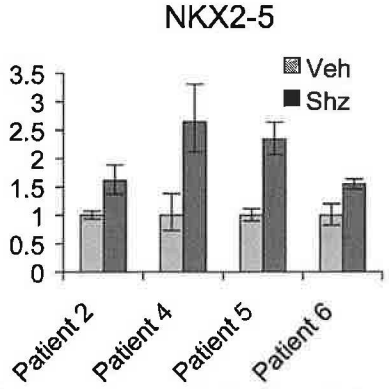


Human bone marrow-derived stem/progenitor cells were pre-treated with cardiogenic sulfonyl-hydrazone synthetic small molecules (and DAPI to label nuclei), and then injected into the injured rat heart. (Left hand panel) Host rat myocardium stained with antibody specific for rat cardiac muscle (green). (Right hand panel) Same myocardium demonstrating colony of drug-treated human stem/progenitor cells stained with human-specific muscle antibody (red) in vicinity of the cell injection site needle track.



Echocardiogram demonstrating improved contractile function in injured rat heart that received small molecule treated human stem/progenitor cells compared to heart that received vehicle treated cells.

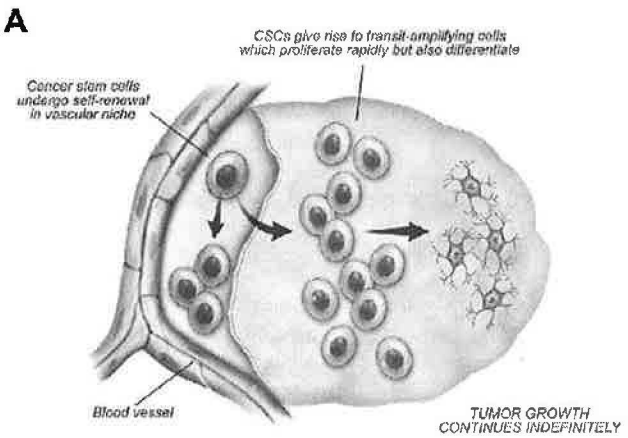
Following-up on these studies, we have also collaborated with colleagues from the German REPAIR-AMI trial and have shown that our sulfonyl-hydrazone and isoxazole small molecules (28, 29), can activate cardiac genes in these clinical-grade bone marrow cells sent to us from Germany (31, 32). These results have immediate translational importance.



Activation of Nkx2.5 cardiac gene by sulfonyl-hydrazone small molecule drug in bone marrow cells from REPAIR-AMI patients following ex vivo treatment. Real time RT-PCR.

Targeting stem/progenitor cells in the niche

The word "niche" comes from the French and literally means *doghouse*. The stem/progenitor cell niche is a highly specialized microenvironment in adult tissues, intimately associated with vascular structures and the circulation. Most stem/progenitor cells reside life-long in the niche, awaiting a call-to-duty. The niche is both an anatomic site and functional domain that nurtures stem/progenitor cells, enabling their self-renewal and, when appropriate, promoting their differentiation. Both self-renewal and differentiation are highly regulated processes, driven by specific extrinsic cues available only in this specialized microenvironment.



Although this is a cartoon of the cancer stem cell niche (2), the fundamental principles are the same for all vascular niches, including the epicardial stem/progenitor niche.

Recent pioneering preclinical work by Dr. David Scadden (Harvard Stem Cell Institute) has begun to develop targeting of the hematopoietic stem/progenitor cell niche as a new mode of therapy. Stem/progenitor cell prodution in the niche is governed by circulating substances, paracrine signals, direct cell-cell contacts, neural inputs, and physical as well metabolic signals from tissue activity (33).

An important concept arising from these early studies is that it is not necessary to target the stem/progenitor cell directly, rather targeting cells that play a supporting role in the niche can lead to improved stem/progenitor cell function, indirectly. Dr. Scadden's group recently reported the first successful targeting of a stem cell niche in vivo (34-37). These are landmark preclinical studies.

Osteoblasts are a type of mesenchymal stem cell in the bone marrow niche with parathyroid hormone seven transmembrane G-protein coupled receptors (38, 39). Activation of PTH receptors on osteoblasts can regulate the number of hematopoietic stem cells. In a translation preclinical study, Scadden and colleagues have now shown that pharmacological treatment of mice with PTH causes increased numbers of stem cells, enhanced tolerance to cytotoxic injury, and improved engraftment efficiency in animal models (35). Circulating PTH activates osteoblasts in the niche that transmit this signal to neighboring hematopoietic stem cells. Exactly how the osteoblasts signals to the hematopoietic stem cell, whether this involves direct cell-cell contact or is mediated by paracrine factors remains to be established.

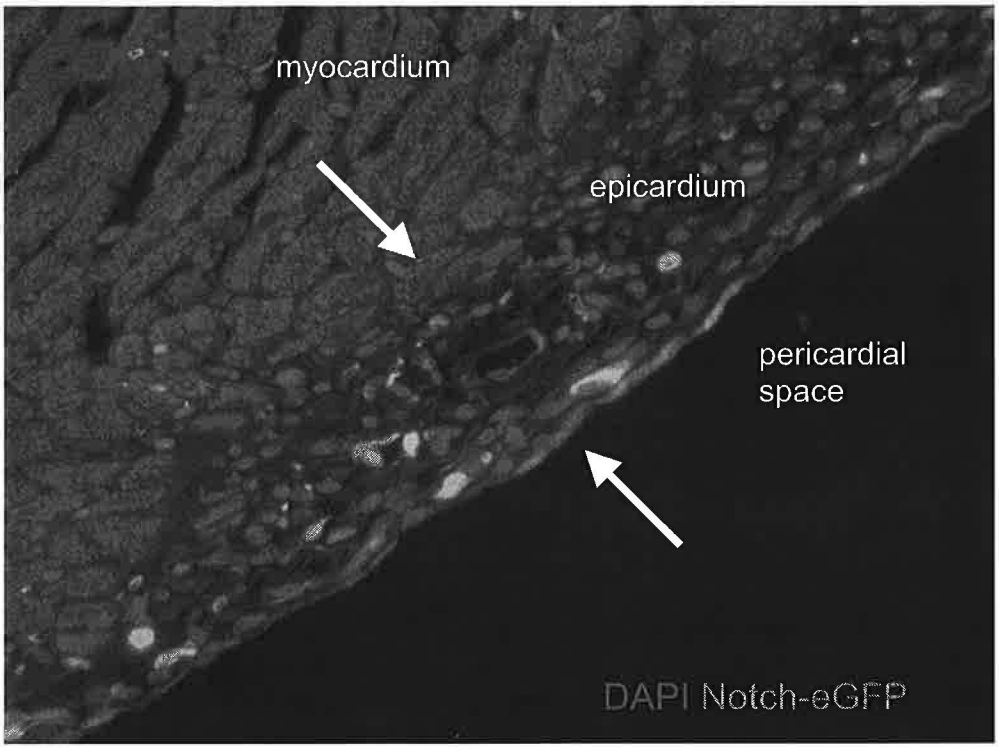
A second study by the Scadden group took advantage of the drug bortezomib, a clinically available proteasome inhibitor used in the treatment of multiple myeloma, which induces mesenchymal stem cells to differentiate into osteoblasts (37). The translational component of this study was to show that ectopically implanted mesenchymal stem cells differentiated into osteoblasts, forming ectopic bone ossicles when graft recipients were treated with low dose bortezomib. Moreover, bortezomib treatment increased bone formation and rescued bone loss in a mouse model of osteoporosis. These studies showed that pharamacological targeting of a stem/progenitor population in vivo increased bone regeneration in mice.

Even more recently and relevant to the heart is a study from an Italian group, demonstrating that PTH augmented the mobilization of hematopoietic stem/progenitor cells by G-CSF (40). Co-treatment with PTH augmented a number of hematologic and physiologic parameters in a mouse model of experimental hindlimb ischemia.

Clinically, in a phase I trial, oncologists at MGH have used PTH to facilitate stem cell mobilization in patients undergoing autologous stem cell transplantation (41). Indeed, PTH rescued stem cell mobilization in almost half of patients who had previously failed to proceed to autologous stem cell transplantation because of inadequate mobilization of hematopoietic stem cells.

Epicardium, lead candidate for the primary stem/progenitor cell niche of the heart

Every clinician knows the epicardium, which is also called the visceral pericardium, the outermost specialized layer of the heart, wedged between the pericardial fluid and the myocardium. All disease processes commonly described as "pericardial" also directly involve the epicardium.



This is an immunofluorescence photomicrograph of epicardium in transgenic Notch reporter mouse used in our studies of epicardial stem/progenitor cells. Nuclei are stained in blue (DAPI) and cells activated in Notch signaling (stem/progenitor cells) are stained in green (eGFP). Arrows demarcate boundaries of epicardial zone.

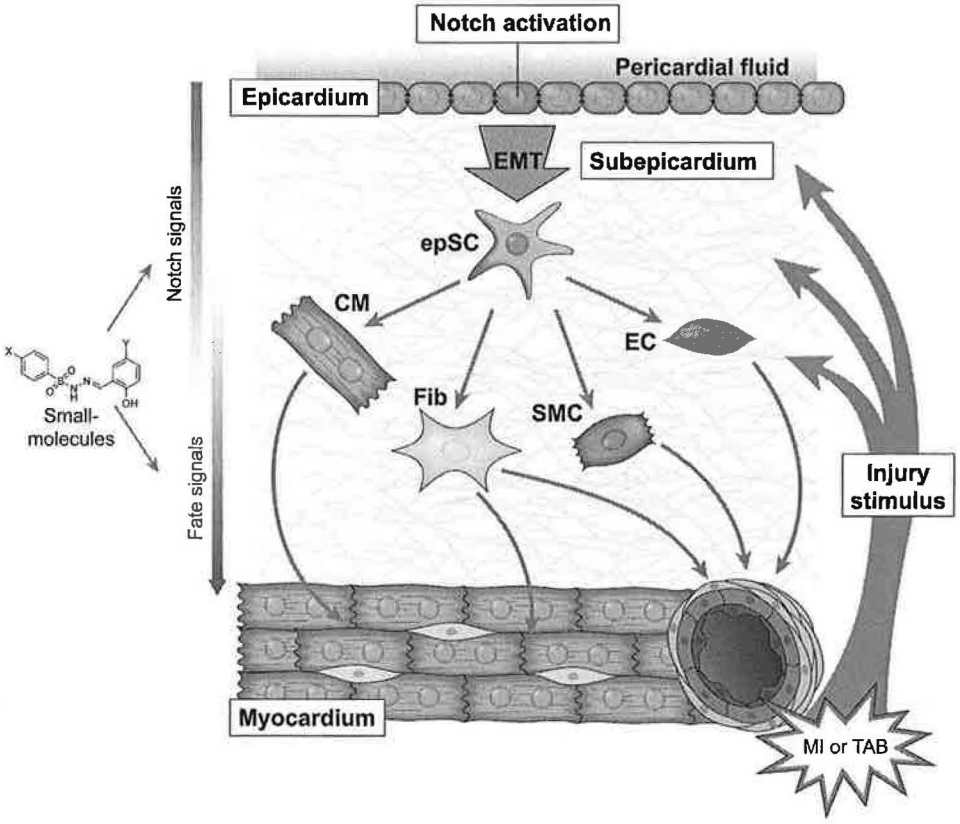
To emphasize the importance of the epicardium/pericardium in medicine, here is a quotation from a classic textbook[4].

"The pericardium is something of an enigma. Like the vermiform appendix, we can very well do without it, and yet when it becomes diseased it can, because of its strategic position, place a stranglehold around the heart and thus threaten life itself. The pericardium has another peculiarity: while seldom the primary seat of disease, it may be involved in almost every disease. In some such instances pericardial involvement overshadows all other features of the systemic disease, because pericardial pain may simulate that of a thoracic catastrophe such as myocardial infarction, massive pulmonary embolism, or dissecting hematoma of the aorta. The pericardial friction rub may be a patient's most dramatic physical sign; pulsus paradoxus never fails to excite the interest of the physician; and the electrocardiographic changes may be alarmingly like those of ischemic disease."

The past few have seen important changes in our understanding of the epicardium/pericardium and it's role in cardiac development, diseases, and repair (15, 42, 43). Indeed, the niche concept provides an entirely new perspective on the epicardium/pericardium and it's role in cancer metastasis, infectious diseases, and collagen vascular diseases. In addition, there is very interesting new data emerging about the adipose tissue layer that covers the human heart. Epicardial adipose tissue is a biologically unique and physiologically and patho-physiologically important fat depot that may originate from epicardial stem/progenitor cells.

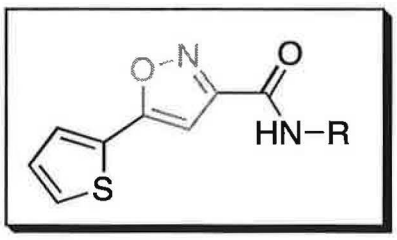
The clinical promise is to pharmacologically "prime" the epicardial stem/progenitor cell niche, increasing the responsiveness of resident cells to injury signals, boosting the delivery of newly born and functionally integrated cardiomyocytes into injured myocardium.

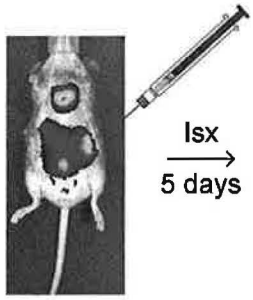
[4] Ralph Shabetai, *The Pericardium*, Grune and Stratton, N.Y., 1981, pg. 1



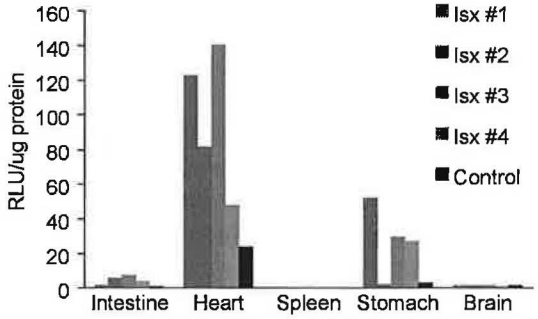
Cartoon model of epicardial stem/progenitor cell niche. Epithelial epicardial cells undergo epithelial-mesenchymal transformation to generate a population of epicardial-derived stem/progenitor cells (epSCs). Epicardial stem/progenitor cells can differentiate into cardiomyocytes (CM), fibroblasts (Fib), smooth muscle cells (SMC), and endothelial cells (EC), which contribute to production of new myocardium and vasculature. Notch pathway signaling plays a key role in adult cardiogenesis.

Targeting the epicardial niche with synthetic small molecules. A major focus of our research program is to target the epicardial niche of the adult heart. We screened the UT Southwestern chemical library for small molecules that activate cardiac genes in stem cells. These molecules activate cardiac genes, mRNAs, miRs, and proteins, in epicardial progenitor cells, and are future therapeutics to enhance the repair/regeneration function of the cardiac stem/progenitor cell niche in vivo.





TNkx2.5^{Luc}R mouse



Isoxazoles, a second class of synthetic small molecules identified in our screen, can activate the cardiac Nkx2.5 gene specifically in the heart (with minor ectopic expression in stomach) of transgenic reporter mice. This small molecule selectively targets epicardial stem/progenitor cells, providing a starting point for the first drugs designed to target the heart's stem/progenitor cell niche.

Summary

Nailed to this wall of eagle baffling mountain,
Ah me! Alas, pain, pain ever, forever!
Heaven's winged hound, polluting from thy lips
His beak, in poison not his own, tears up my ***heart***.
Prometheus Unbound. Percy Bysshe Shelley, 1792-1822

The next frontier for cardiovascular medicine is to bring the Prometheus dream to fruition. The time is rapidly approaching where clinicians may be managing stem/progenitor cells as a general therapeutic strategy. Drugs that enhance the function of the niche, promoting growth, differentiation, and survival of critical cell types, may be the key to making the dream a reality.

And, finally, a modern stem cell classic:

Mama don't let your stem cells grow up to be cowboys

Mama don't let your stem cells grow up to be cowboys
Don't let 'em pick guitars and drive them old trucks
Make 'em be muscle and neurons and such
Mama don't let your stem cells grow up to be cowboys
They'll never stay home and they're always alone
Even in niches they love
Stem cells ain't easy to love and they're harder to grow
And they'd rather give tumors then diamonds or gold
...
And if you don't understand 'em and they don't die young
They'll probably just differentiate away...

Annie and Willie Nelson Professor of Stem Cell Biology, UT Southwestern (44)

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